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Soil Enzyme Kinetics, Temperature Sensitivity and the Response of Soil Enzymes to Tillage and Conservation-Based Management: A Review

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***Abstract*—** *Soil enzymes mediate the breakdown of organic matter and the release of carbon, nitrogen and phosphorus, and their activity is widely used as an early indicator of changes in soil quality. Interpreting that activity, however, requires an understanding of both the kinetics of the enzymes themselves and the environmental and management conditions under which they operate. This narrative review draws together recent studies and reviews on three linked themes: the kinetic description of enzyme-catalyzed reactions and the way pH, temperature and inhibitors modify it; the temperature sensitivity of soil enzymes and its consequences for nutrient stoichiometry; and the response of enzyme activities to tillage, residue retention and climate-smart cropping systems, including the role of dehydrogenase as an indicator of microbial function. The evidence shows that kinetic parameters measured in soil are apparent rather than intrinsic, that temperature sensitivity differs strongly among enzyme groups and can fall below the commonly assumed Q10 of 2, and that reduced tillage and residue retention generally raise soil organic carbon, microbial biomass and the activity of many hydrolytic enzymes, although not all enzymes respond and the relationships between enzymes and environmental factors change with management. Most field studies report potential activity from a single site and season and do not measure kinetic parameters, which limits mechanistic interpretation. Studies that combine kinetic parameters, temperature responses and management treatments across seasons are needed.*

***Keywords*— soil enzymes, enzyme kinetics, Michaelis–Menten, temperature sensitivity, tillage, conservation agriculture, dehydrogenase, soil quality.**

I. INTRODUCTION

Most of the organic matter in soil is present as polymers that microorganisms cannot take up directly. Microbes therefore release enzymes that depolymerize this material into soluble oligomers and monomers, which are then transported into the cell (Wallenstein et al., 2011). Enzymes that release glucose, amino acids and phosphate, such as β-glucosidase, aminopeptidases and phosphatases, sit at the interface between soil organic matter and microbial nutrition, and their activity influences both nutrient availability and the fate of carbon. Because enzyme activity responds to disturbance and to changes in management sooner than many bulk soil properties, it has been proposed as a sensitive indicator of soil quality (Pandey et al., 2015; Jat et al., 2021).

At the same time, agricultural systems are being redesigned to produce more food with less degradation of the soil. Conservation agriculture (CA) is one such approach. It combines minimum or no tillage with permanent soil cover and diversified crop rotations that include legumes, and it is promoted as a means of maintaining soil organic carbon and soil function (Page et al., 2020). Page et al. (2020) concluded that the effect of CA on soil organic carbon varies among regions, but that where conditions favor biomass production and yields are not penalized, CA generally leads to higher organic carbon

near the soil surface, often accompanied by better soil structure and greater microbial biomass and diversity. Enzyme activities are a natural way to probe whether these changes in organic carbon also translate into changes in biological function.

Two bodies of research address enzymes in soil, and they are often reported separately. Biochemical and ecological studies describe how fast enzyme-catalyzed reactions proceed and why, using the language of kinetic parameters and activation energies (Ahenkorah et al., 2021; Wallenstein et al., 2011; Bárta et al., 2013). Agronomic studies compare enzyme activities among tillage systems, cropping systems and land uses (Pandey et al., 2015; Jat et al., 2021; Kumar et al., 2013). The purpose of this review is to bring these two perspectives together. We first set out the kinetic framework and the factors that modify it, then examine temperature sensitivity, then summarize evidence on tillage and conservation-based management, and finally consider dehydrogenase as a general indicator. We close by identifying the main limitations of current evidence and the research needed to link kinetics with management. The review is based mainly on six recent studies and reviews (Table 1), supplemented by earlier work cited within them and clearly attributed where used.

TABLE 1
PRINCIPAL SOURCES USED IN THIS REVIEW

Source	System or scope	Focus	Main findings used here
Ahenkorah et al. (2021)	Review; jack bean urease in enzyme-induced carbonate precipitation	Michaelis–Menten kinetics; pH, temperature and product inhibition	pH acts mainly on Vmax; ammonium is a non-competitive inhibitor; a combined rate equation was proposed but tested on few data sets
Wallenstein et al. (2011)	Book chapter; soil enzymes generally	Temperature sensitivity, apparent kinetics, in situ activity	Parameters in soil are apparent; Q10 of enzymes often below 2; temperature can matter more than enzyme pool size for in situ rates
Bárta et al. (2013)	Acidic spruce forest soil, Central Europe; litter and organic horizons	Kinetics and temperature response of five enzymes; modeled seasonal activity	Km and Vmax differ between horizons; aminopeptidases insensitive or negatively sensitive to warming; modeled C:N acquisition shifts seasonally
Pandey et al. (2015)	Long-term rice–wheat experiment, Varanasi, India; four tillage combinations	Microbial biomass and six enzymes during one wheat season	Biomass and most hydrolases higher under reduced tillage; polyphenol oxidase opposite; enzyme–environment relationships differ among systems
Jat et al. (2021)	Cereal systems under conventional, conservation and climate-smart management, Karnal, India; rhizosphere and bulk soil	Eight enzymes, organic carbon, enzyme indices	Many enzymes higher under conservation systems; urease unchanged; rhizosphere–bulk differences depend on enzyme and stage
Kumar et al. (2013)	Review; natural, degraded and mine soils	Dehydrogenase activity and its measurement	Activity higher in undisturbed soils and lower in polluted or eroded soils; strongly method-dependent
Page et al. (2020)	Narrative review; conservation agriculture worldwide	Soil organic carbon and soil properties	Used for the definition of CA and the context of organic carbon change

Only studies discussed in detail are listed.

II. KINETIC FRAMEWORK FOR SOIL ENZYMES

2.1 Michaelis–Menten Kinetics and Apparent Parameters

The quantitative description of enzyme catalysis rests on the model of Michaelis and Menten (1913). In this model, the enzyme and substrate form a complex that either dissociates or proceeds to product, and under a quasi-steady-state assumption the reaction velocity rises hyperbolically with substrate concentration toward a maximum (Vmax). The substrate concentration that gives half of this maximum is the Michaelis constant (Km), which is often read as an index of how strongly the enzyme binds its substrate. Vmax is the product of the catalytic rate constant and the total enzyme concentration, and the ratio of kcat to Km is commonly used to express catalytic efficiency (Ahenkorah et al., 2021).

The quasi-steady-state approximation has limits. Ahenkorah et al. (2021) summarize earlier work showing that it is reliable only when the substrate greatly exceeds the enzyme, with one criterion cited being a substrate-to-enzyme ratio above about 100 (Stayton and Fromm, 1979), and that modified, integrated rate equations are needed at high enzyme concentrations. A second set of qualifications applies in soil. Wallenstein et al. (2011) note that when the model is applied to whole soils the assumptions underlying it are not met, and the fitted parameters are better described as apparent Vmax and apparent Km. Apparent Vmax then reflects, to a first approximation, the amount of active enzyme, which in soil is a mixture of isoenzymes from many organisms. Apparent Km is influenced by the background pool of natural substrate, which competes with the artificial substrate used in the assay (Chróst, 1990; Wallenstein et al., 2011). A difference in apparent Km between two soils or horizons is therefore not necessarily evidence of a structurally different enzyme.

2.2 Estimation of Kinetic Parameters

Kinetic parameters can be obtained by fitting the hyperbolic equation directly by nonlinear regression or by using linear transformations such as the Lineweaver–Burk, Hanes–Woolf and Eadie–Hofstee plots. Each linearization distorts the error structure of the data in a different way, and the comparison in Ahenkorah et al. (2021) points, for example, to large errors at low substrate concentration in the double-reciprocal plot. The same review distinguishes discontinuous assays, in which product is measured once after a fixed time, from continuous assays in which product formation is followed over time. According to the authors, most optimization studies of enzyme-induced carbonate precipitation have used discontinuous measurement, which cannot separate the effect of enzyme activity from that of product inhibition and which helps to explain why reported optimum reagent concentrations differ among laboratories (Ahenkorah et al., 2021).

In soil enzymology, a further practical point applies. Many studies use fluorogenic or colorimetric analog substrates at a single incubation temperature, for example in microplate fluorometric assays (Marx et al., 2001; Bárta et al., 2013), and so estimate potential activity under non-limiting substrate rather than the rate occurring in the field (Wallenstein et al., 2011).

2.3 Effects of pH, Temperature and Inhibitors

Ahenkorah et al. (2021) provide the most explicit treatment of how environmental modifiers enter the rate equation, using urease from jack bean as an example. Based on earlier experimental data (Fidaleo and Lavecchia, 2003), they describe pH effects through the dissociation constants of the free enzyme and of the enzyme–substrate complex. In the data they analyze, Vmax is greatest near neutral pH and declines sharply on either side, whereas Km varies comparatively little, so that pH acts mainly on the catalytic step. Temperature is treated as acting through kcat according to an activation energy, which they report in the range of roughly 33–36 kJ mol^{-1} for urea hydrolysis. The reaction product, ammonium, behaves as a non-competitive inhibitor; in the data set they use, the fitted values were a kcat of about 18 min^{-1}, a Km of about 3.2 mmol L^{-1} and an apparent inhibition constant of about 12 mmol L^{-1} (Ahenkorah et al., 2021).

The authors combine these terms in a single modified rate equation and compare it with published urease data across a range of temperatures and with one data set on calcium release during carbonate precipitation. The fit was good but imperfect, and the deviations were attributed to unreported inhibitors or pH change (Ahenkorah et al., 2021). The strength of the approach lies in showing that pH, temperature and inhibition can be treated together; its weakness, acknowledged by the authors, is the small number of suitable data sets, most of them from jack bean urease. Although the application is an engineering one, the same classes of modifier are relevant in soil. Ammonium accumulation, buffer chemistry, heavy metals and phenolic compounds

may all alter apparent kinetics, and Bárta et al. (2013) invoke inhibition by phenolics and by N-rich compounds to explain kinetic differences between forest soil horizons (see below). Krajewska (2009) provides a broader review of the catalytic and kinetic properties of ureases.

III. TEMPERATURE SENSITIVITY OF SOIL ENZYMES

3.1 Activation Energy and Q10

The temperature dependence of a reaction rate is usually described by the Arrhenius relationship, in which rate increases exponentially with temperature at a rate set by the activation energy (Ea). A rate that doubles for every 10 °C rise (Q10 = 2) corresponds to an Ea of about 0.5 eV, or roughly 48 kJ mol^{-1} (Wallenstein et al., 2011). Wallenstein et al. (2011) emphasize that two different properties are frequently conflated: the thermal stability of the protein, which depends on its overall folded structure, and the temperature sensitivity of catalysis, which depends on the flexibility of the active site. The active site tends to lose function at temperatures lower than those needed to denature the whole protein. Cold-adapted enzymes generally have more flexible active sites and lower activation energies, while heat-adapted enzymes have more rigid ones.

In soil these molecular distinctions are blurred, because a named enzyme such as β-glucosidase is in reality a population of isoenzymes of different origin, some of which may be sorbed to mineral or humic surfaces that restrict conformational flexibility. Estimates of Ea and optimum temperature then become statistical distributions that depend on the scale of comparison (Wallenstein et al., 2011). These authors give about 0.3 eV as a typical Ea for extracellular enzymes that are not conformationally constrained, in contrast to about 0.62 eV for microbial metabolism generally (Gillooly et al., 2001), which would imply that the production of enzymes by microorganisms can respond more strongly to temperature than the catalytic step does. Measured Q10 values for soil enzymes are also often below 2. In a study of nine enzymes in three grassland soils, only β-glucosidase in one soil exceeded a Q10 of 2 at 20 °C, and most enzymes were closer to 1.5 (Trasar-Cepeda et al., 2007; Wallenstein et al., 2011).

Q10 is an empirical summary rather than a mechanism, and it changes with temperature, so it cannot be extrapolated reliably beyond the range in which it was measured (Wallenstein et al., 2011). An alternative favored in the literature is a mechanistic description in which both Vmax and Km depend on temperature. Because Km tends to rise with temperature, reducing substrate affinity, this effect can partly or wholly cancel the gain in Vmax when substrate concentrations are close to Km (Davidson and Janssens, 2006; Wallenstein et al., 2011).

3.2 Direct and Indirect Effects of Temperature

Temperature also acts indirectly on enzyme activity. It changes microbial carbon-use efficiency, the rate at which enzymes are produced and degraded, and the availability of substrate, since desorption from mineral surfaces increases with warming (Wallenstein et al., 2011). Because laboratory assays are generally run with excess substrate, they may underestimate the temperature sensitivity of in situ activity. The same authors caution that the temperature response of heterotrophic respiration need not mirror that of extracellular enzymes, because the products of enzyme action must still be transported and metabolized before CO_2 is released.

A central argument of that chapter is that measuring potential activity at a single reference temperature neglects the influence of temperature on activity in the field, where soil temperature varies over much wider ranges than enzyme pools do. In an Arctic tundra study cited by the authors, temperature explained about 72% of the variation in modeled in situ β-glucosidase activity, more than seasonal changes in the enzyme pool (Wallenstein et al., 2009). In the same study, enzymes degrading N-rich compounds had a lower mean Q10 than those degrading carbon compounds, so that warming alone, with no change in enzyme pools, would favor carbon mineralization relative to nitrogen mineralization (Wallenstein et al., 2009; Wallenstein et al., 2011).

3.3 Evidence from an Acidic Forest Soil

Bárta et al. (2013) tested these ideas in a Norway spruce forest soil in the Bohemian Forest, comparing a litter horizon (0–2 cm) and an organic horizon (5–10 cm). They measured the kinetics and temperature response of β-glucosidase,

cellobiohydrolase, two aminopeptidases and phosphatase, and combined the fitted temperature responses with continuous in situ temperature records to model potential activity through a growing season (Table 2).

TABLE 2
APPARENT KINETIC PARAMETERS AND TEMPERATURE SENSITIVITY OF FIVE HYDROLYTIC ENZYMES IN THE LITTER (L) AND ORGANIC (O) HORIZONS OF AN ACIDIC SPRUCE FOREST SOIL (MEAN VALUES; ADAPTED FROM BÁRTA ET AL., 2013)

Enzyme	Vmax (nmol h^{-1} g C^{-1}), L / O	Km (µM), L / O	Q10 (0–25 °C), L / O	Ea (kJ mol^{-1}), L / O
β-Glucosidase	597.5 / 106.0	2.5 / 0.5	1.67 / 1.61	45.7 / 43.8
Cellobiohydrolase	42.9 / 4.2	0.6 / 0.3	0.72 / 0.69	not determined
Alanine-aminopeptidase	38.7 / 73.2	5.9 / 281.5	0.66 / 0.64	not determined
Leucine-aminopeptidase	47.6 / 54.5	13.7 / 549.7	0.62 / 0.51	not determined
Phosphatase	505.5 / 82.8	27.7 / 6.9	1.50 / 1.74	37.9 / 46.7

Differences between horizons were not significant for all parameters; see Bárta et al. (2013) for the statistical comparison.

The results illustrate several of the points made above. First, kinetic parameters differed strongly between horizons, and in opposite directions for different enzyme groups. Both aminopeptidases had a much lower Km in the litter than in the organic horizon (for alanine-aminopeptidase, roughly 6 versus 280 µM) together with higher catalytic efficiency, whereas phosphatase and cellobiohydrolase had lower Km values in the organic horizon. The authors suggest that litter peptidases act efficiently at low peptide concentrations, so that part of the complex dissolved organic nitrogen might pass undegraded to the lower horizon, where it could be lost by leaching (Bárta et al., 2013). Given the caveat on apparent Km above, this interpretation depends on the differences reflecting real differences in isoenzyme pools, competitive inhibition or enzyme–phenolic complexes, which the authors discuss but do not test directly.

Second, temperature responses were far from uniform. β-Glucosidase and phosphatase followed Arrhenius behavior, with activation energies of about 38–47 kJ mol^{-1} and Q10 values of roughly 1.5–1.7. By contrast, the aminopeptidases and cellobiohydrolase showed no increase, or even a decline, with warming between 0 and 25 °C, giving Q10 values below one, which the authors state had not previously been reported for soil enzymes (Bárta et al., 2013). They propose several explanations: the very low soil pH of about 3, which may affect activation energy (compare Steinweg et al., 2013); a cold-adapted microbial community in soil sampled in autumn; and complexation of enzymes with the abundant phenolic compounds derived from spruce needles. These remain hypotheses.

Third, because carbon-acquiring enzymes warmed while nitrogen-acquiring enzymes did not, the modeled ratio of carbon to nitrogen acquisition activity rose in summer, and the modeled available C:N ranged from about 12 to 34 in the litter over the season. In the organic horizon the activity ratio remained below a threshold value (3.2) proposed to mark a shift toward nitrogen-limited metabolism, and the carbon-to-phosphorus activity ratio indicated phosphorus limitation in both horizons (Bárta et al., 2013; Allen and Gillooly, 2009; Sinsabaugh et al., 2009). The model reproduced the direction of seasonal change but not its magnitude: modeled activity ratios reached about 76% and 54% of the measured C:P and C:N ratios, respectively. Soil moisture was omitted from the model, which the authors justify by the narrow range of moisture at the site.

IV. RESPONSE OF SOIL ENZYMES TO TILLAGE AND CONSERVATION-BASED MANAGEMENT

4.1 Tillage Systems in Rice–Wheat Cropping

Pandey et al. (2015) studied a long-term field experiment, begun in 2003 at Varanasi, India, in which four combinations of conventional tillage and no tillage were applied to rice and wheat. During the 2010–2011 wheat season they sampled every five days and measured microbial biomass carbon and nitrogen together with six enzymes: β-glucosidase, cellobiohydrolase, polyphenol oxidase, alkaline phosphatase, glycine-aminopeptidase and urease. Enzyme assays were incubated at the in situ soil temperature at the time of sampling. By 2010 soil organic carbon had increased from about 0.38% under continuous conventional tillage to about 0.96% under continuous no tillage, with intermediate values under the mixed systems, while bulk density was highest under continuous no tillage (Pandey et al., 2015).

Microbial biomass was highest under continuous no tillage and lowest under continuous conventional tillage, and most hydrolytic enzymes were more active with reduced tillage. The ranking among systems, however, was not simply a function of tillage frequency. β-Glucosidase activity was usually greatest in the system with no tillage before rice and tillage before wheat, which the authors attribute to the mixing of wheat residues into the soil. Polyphenol oxidase was the only enzyme to decline as tillage was reduced, in line with the general inverse relationship between organic carbon and phenol oxidase activity noted by Sinsabaugh (2010). Temporal patterns also differed among enzymes: urease and glycine-aminopeptidase followed nitrogen demand and fertilizer timing, and alkaline phosphatase rose late in the season, which the authors link to phosphorus demand (Pandey et al., 2015). The general tendency for enzymes to be more active under no tillage agrees with earlier long-term work (Dick, 1994).

Pandey et al. (2015) also tested whether the relationships between enzymes and environmental variables were consistent among tillage systems, using curve fitting and principal component analysis of enzyme activities, soil temperature, water content and redox potential. They concluded that they were not. The form, and in some cases the significance, of relationships among particular enzymes and between enzymes and temperature or moisture varied among systems; for example, glycine-aminopeptidase increased linearly with soil temperature in three systems but exponentially under conventional tillage. This finding connects directly to the kinetic literature discussed above, because it indicates that the apparent temperature sensitivity of an enzyme in the field is not a fixed property but depends on the soil conditions that management creates.

4.2 Residue Retention, Crop Rotation and Climate-Smart Systems

Jat et al. (2021) compared conventional rice–wheat with partial and full conservation-based systems at Karnal, India, in an experiment established in 2009. The conservation systems combined zero tillage, residue retention and mungbean in the rotation, and the fully climate-smart treatments added subsurface drip irrigation and precisely placed nitrogen. Both rice-based and maize-based variants were included. Sampling in the 2017–2018 wheat season covered four crop stages, and eight enzymes (dehydrogenase, β-glucosidase, acid and alkaline phosphatase, fluorescein diacetate hydrolase, arylsulfatase, urease and cellulase) were measured together with soil organic carbon, moisture and three derived indices.

Averaged across stages and sampling zones, β-glucosidase activity was about 20% higher under the conservation-based scenarios than under conventional tillage, arylsulfatase was about 28% higher, and cellulase activity roughly doubled (about 15.5 versus 8.0 μg glucose g^{-1} h^{-1}). Urease, in contrast, did not differ between the conservation-based treatments and conventional tillage. Rice-based systems showed higher fluorescein diacetate hydrolase activity than maize-based systems (Jat et al., 2021). The authors relate these patterns to residue load, which reached about 111 Mg ha^{-1} over eight years in the rice-based climate-smart systems and about 129 Mg ha^{-1} in the maize-based ones, and to differences in residue quality: maize roots and stover have a lower C:N ratio than rice, which the authors suggest favors faster decomposition (Jat et al., 2021). An earlier study from the same region, as reported by these authors, found much higher dehydrogenase and alkaline phosphatase activity under maize–wheat–mungbean and rice–wheat–mungbean systems with residue retention than under conventional rice–wheat (Choudhary et al., 2018).

4.3 Rhizosphere and Bulk Soil

A distinctive feature of the study by Jat et al. (2021) is the comparison between rhizosphere and bulk soil. The rhizosphere is generally regarded as a hot spot of microbial activity because of rhizodeposition (Kuzyakov, 2002; Pausch and Kuzyakov, 2018), but the results showed that the difference depended on the enzyme, crop stage and system. Some enzymes were more active near roots before sowing, while others were as active or more active in bulk soil, especially where residues were retained at the surface. For example, dehydrogenase activity after harvest in rice-based climate-smart systems was about 21% higher in bulk soil than in rhizosphere soil. The authors attribute such patterns to labile carbon released from decomposing surface residues and to volatile organic compounds that may be carried away from roots, and they conclude that conservation-based management improves enzyme activity in bulk soil as well as in the rhizosphere.

4.4 Enzyme Indices and Their Relationships

Jat et al. (2021) also condensed their data into three indices. The ratio of alkaline to acid phosphatase, proposed as an enzymatic indicator of soil pH, ranged from 0.71 to 1.34 and was negatively correlated with β-glucosidase activity, soil organic carbon and residue load. An alteration index based on β-glucosidase, phosphatase and urease (Puglisi et al., 2006) was lowest, indicating better soil quality, at intermediate crop stages and in conservation-based systems. The geometric mean of all enzyme activities was positively correlated with soil organic carbon ($r = 0.93$), soil moisture and residue load, and was highest in the

partial conservation-based system at maximum tillering and flowering. The authors interpret these results as evidence that residue retention raises overall enzymatic function. Such indices are convenient, but they weight each enzyme equally and inherit any inconsistency in the underlying assays.

4.5 Points of Agreement and Divergence

The two field studies reach compatible but not identical conclusions. Both report higher organic carbon and higher activity of many carbon-, nitrogen- or phosphorus-cycling enzymes under reduced-tillage or conservation systems, which agrees with the broader pattern in which conservation management tends to raise surface soil organic carbon where biomass production is favorable (Page et al., 2020). Both also show that the response is enzyme-specific: polyphenol oxidase moved in the opposite direction to the hydrolases in Pandey et al. (2015), and urease did not respond to management in Jat et al. (2021). This is consistent with the kinetic literature, which argues that enzymes controlled by different substrates and demands should not be expected to respond uniformly (Wallenstein et al., 2011; Bárta et al., 2013). Both studies also report that effects change with crop stage, so a single sampling date is not a safe basis for ranking systems.

The studies differ in what they take enzyme activity to indicate. Pandey et al. (2015) place weight on relationships among enzymes and between enzymes and environmental variables, and use the lack of consistency in those relationships as a principal finding. Jat et al. (2021) emphasize composite indices and the distinction between rhizosphere and bulk soil. Neither measured kinetic parameters, so neither can distinguish a larger enzyme pool (higher Vmax) from a change in substrate affinity (Km) or in temperature response. This is the main gap between the agronomic and the kinetic literatures.

V. DEHYDROGENASE AS AN INDICATOR OF MICROBIAL FUNCTION

Dehydrogenase differs from the hydrolases discussed above because it is intracellular and is regarded as reflecting the oxidative metabolism of living microorganisms rather than a pool of accumulated enzyme (Kumar et al., 2013). In their review, Kumar et al. (2013) describe two main families of assay. One is based on reduction of triphenyltetrazolium chloride (TTC) to a red formazan (Casida et al., 1964), and the other on reduction of iodonitrotetrazolium chloride (INT) (Von Mersi and Schinner, 1991). Several authors cited in the review judged INT to be the more accurate substrate, because its higher electron affinity allows it to compete better with oxygen and so reduces the need for anaerobic incubation (Trevors, 1984; Kumar et al., 2013). Results depend strongly on incubation time and temperature, pre-incubation, aeration, moisture and pH, and the review notes that formazan production stops being linear after roughly 9–12 hours, so that the 24-hour incubations that are commonly used are the part of the method most likely to cause problems of interpretation.

With these caveats, the review collates evidence that dehydrogenase activity is higher in natural than in degraded or polluted soils, decreases along erosion gradients on mine spoil, is positively related to soil organic carbon and moisture, and differs among plant species used for revegetation. In a heavy-metal-contaminated site, for example, activity fell steeply with increasing pollution and responded less to rewetting (Hinojosa et al., 2004; Kumar et al., 2013). These observations support the use of dehydrogenase as a sensitive indicator of disturbance, and Jat et al. (2021) similarly found that dehydrogenase varied with management system, crop stage and sampling zone.

A practical difficulty visible in the summary table of Kumar et al. (2013) is that results are reported with different substrates and units (for example nmol, µg and mg of INTF or TPF per gram of soil per hour, and in one case pkatal), so values from different studies cannot be compared directly. The review is also descriptive and does not weigh the quality of the studies it summarizes.

VI. LINKING KINETICS, ENVIRONMENT AND MANAGEMENT

Three themes connect the studies reviewed here. The first is that enzyme activity is the joint outcome of pool size, substrate affinity and temperature response. The kinetic literature separates these as Vmax, Km and Ea (Wallenstein et al., 2011; Ahenkorah et al., 2021; Bárta et al., 2013), whereas management studies usually report a single activity at one temperature, which conflates them. The higher β-glucosidase activity under conservation systems in Pandey et al. (2015) and Jat et al. (2021) could reflect more enzyme, more available substrate or a different temperature regime, and in the first study the no-tillage soil was cooler than the tilled soil. Measuring Vmax and Km at several temperatures, as in Bárta et al. (2013), would allow these explanations to be separated.

The second theme is that enzyme groups respond differently, which changes the stoichiometry of nutrient acquisition. Both the temperature-sensitivity data (Wallenstein et al., 2011; Bárta et al., 2013) and the management data (Pandey et al., 2015; Jat et al., 2021) indicate that carbon-, nitrogen- and phosphorus-acquiring enzymes do not move together. Bárta et al. (2013) show how this can shift the carbon-to-nitrogen balance of potential nutrient release across a season, and Pandey et al. (2015) show that correlations among enzymes change with tillage regime. This matters for ecoenzymatic stoichiometry, in which ratios of carbon-, nitrogen- and phosphorus-acquiring enzyme activities are interpreted as indicators of microbial nutrient demand (Sinsabaugh et al., 2009). The studies reviewed here suggest that such ratios are themselves dependent on temperature and management and should be interpreted in light of the conditions of measurement.

The third theme is that composite indicators are useful but depend on what is included and how it is measured. The geometric-mean and alteration indices used by Jat et al. (2021) correlated with soil organic carbon and residue load, and the dehydrogenase literature shows how strongly assay choice can influence absolute values (Kumar et al., 2013). Standardized assays and reporting of the underlying kinetic conditions would make such indices more comparable among studies.

VII. LIMITATIONS AND FUTURE RESEARCH NEEDS

Several limitations constrain what the current evidence supports. First, most studies measure potential activity under assay conditions rather than activity in the field. Pandey et al. (2015) incubated assays at the in situ soil temperature, which is informative, but this still does not capture substrate limitation in the field. Wallenstein et al. (2011) caution that in situ activity cannot at present be measured directly and that modeling is the available route, with considerable uncertainty.

Second, replication in time and space is limited. The management studies each relate to one site and, for the sampling reported, one wheat season (Pandey et al., 2015; Jat et al., 2021). In Bárta et al. (2013) the kinetic and temperature-sensitivity measurements come from soil sampled at one time, in October, with three replicates, so that seasonal changes in the enzyme community, which the authors themselves suggest could alter temperature response, were not measured directly. In Pandey et al. (2015) the samples were drawn from three plots per treatment, which may limit the independence of observations in the statistical tests.

Third, much of the interpretation is correlative. Explanations such as differences in residue quality, priming, phenolic inhibition or cold-adapted communities are generally not tested experimentally (Pandey et al., 2015; Jat et al., 2021; Bárta et al., 2013). Correlations in Jat et al. (2021) are calculated across pooled scenarios and sampling stages and may therefore reflect shared covariation among treatment means rather than independent relationships. Fourth, the modified rate equation of Ahenkorah et al. (2021) was evaluated against only a small number of published data sets, largely for jack bean urease, so that its transfer to soil enzymes is an extrapolation. Finally, differences in substrates, units and incubation conditions prevent quantitative synthesis across studies, as is especially evident for dehydrogenase (Kumar et al., 2013).

Future work would benefit from (i) measuring Vmax, Km and Ea together within management experiments, preferably across seasons; (ii) combining temperature and moisture in mechanistic models of in situ activity; (iii) experimental tests of proposed explanations such as phenolic inhibition and pH effects on activation energy; and (iv) the standardization of assay protocols, especially for dehydrogenase.

VIII. CONCLUSIONS

Enzyme kinetics offers a quantitative way of separating the effects of enzyme quantity, substrate affinity and environmental modifiers such as pH, temperature and inhibitors, but parameters measured in soil are apparent ones and must be interpreted with care. Temperature sensitivity is enzyme-specific and context-dependent: in an acidic forest soil, hydrolytic enzymes for carbon and phosphorus warmed as expected whereas aminopeptidases did not, with consequences for modeled nutrient release. Reduced tillage and residue-retaining systems generally raise soil organic carbon, microbial biomass and the activity of many carbon-, nitrogen- and phosphorus-cycling enzymes, but not all enzymes respond, and the relationships between enzymes and environmental variables change with management. Dehydrogenase remains a useful but method-sensitive indicator of overall microbial function in disturbed and natural soils. Linking these strands will require studies that measure kinetic parameters directly in managed soils across seasons, using standardized methods.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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